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Genetics

Targeted gene mutations in *Drosophila*

(cDNA/*P* element/polymerase chain reaction/photoreceptor)

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ABSTRACT **A cloned gene can be of interest because of its expression in a particular tissue or at a certain developmental stage, or because of homology to an interesting gene from another organism. In *Drosophila* its location in the genome is readily determined by *in situ* hybridization to the banded larval salivary gland polytene chromosomes, but it is more difficult to isolate mutations that may reveal its function. This paper describes a general method for detecting transposable element insertions into the gene in question. This "reverse genetics" then offers the possibility of observing a consequent mutant phenotype, providing a key to the normal function of the gene. The sensitivity of the polymerase chain reaction makes it possible to detect the occurrence of a single appropriate *P*-element transposon insertion among a population of mutagenized flies. This is accomplished by the use of oligonucleotide primers—one a sequence from within the cloned gene and the other homologous to the terminal sequence of the *P*-element DNA—to prime synthesis into the DNA flanking an insertion site. A segment of DNA, bounded by the two primers, will be a target for amplification only in a fly in which a *P*-element has inserted within about 2 kilobases of the gene primer. This technique has been used to detect *P*-element insertions near a gene expressed in the *Drosophila* compound eye. Potential problems with the technique and possible refinements in the screen are discussed. In principle, it could be utilized to detect insertion of a foreign element into any gene for which at least a partial sequence is known and could be extended to other organisms.**

The polymerase chain reaction (PCR) permits the geometric amplification of a single template molecule through the use of two oligonucleotide primers that flank a segment of DNA that can range in length from 20 to 2500 nucleotides (1, 2). It involves heat denaturation of the DNA, hybridization of the primers, and extension of the primers with a heat-stable DNA polymerase [*Thermus aquaticus (Taq)* polymerase]. The cycle is repeated 40–50 times, resulting in exponential amplification of the target DNA molecule, whereas DNA adjacent to but not flanked by primers is replicated only linearly. This technique is extremely sensitive, allowing for the amplification of a single virus molecule in a background of 10^5 mammalian cells, yielding nanogram quantities of the amplified sequence (2).

Fig. 1 illustrates the method used in this study. Oligonucleotide primers complementary to a single strand of the target gene were included with an oligonucleotide that primed synthesis from the terminal repeats of the transposable *P* element in DNA flanking an insertion site. A substrate suitable for amplification would arise only if a transposition event had led to the insertion of a *P* element within about 2 kilobases (kb) from one of the gene oligonucleotides, creating a segment of DNA appropriately flanked by primers.

Salient features of the method include the ability to detect insertions in the heterozygous state and the ability to screen a mutagenized population with various sets of oligonucleotides corresponding to different genes or to different regions within one gene. The screen for insertions involved the mobilization of multiple transposition-defective *P* elements through the transient introduction of high levels of transposase activity (4). The mutagenized flies, each carrying an average of about 10 new sites of *P*-element insertion (4), were mated to flies carrying multiple inversions of the third chromosome (to suppress crossing over) and subsequently were screened in groups of about 100 for insertions near a gene oligonucleotide. When an insert was found within a particular set of flies, their progeny were progressively subdivided and rescreened until a line of flies, each of which carried the insert, was established.

MATERIALS AND METHODS

Mutagenesis. Flies were maintained on cornmeal/yeast medium (5) in a humidified 25°C room except as noted. To obtain *P*-element insertions, the dysgenic procedure of Robertson *et al.* (4) was used. This makes use of a strain of flies carrying on the second chromosome 17 defective *P* elements, which are mobilized by crossing with a second strain that provides transposase activity from an element on the third chromosome that cannot itself transpose. Two stocks of Birm-2; ry^{506}, isogenic for both the second and third chromosomes, provided the "ammunition" for the mutagenesis, while two stocks isogenic for the second chromosome (iso-2; *Sb* ry^{506}*P*[ry^+2-3](99B)/TM6B, *Hu Dr*) provided the transposase. These stocks were used in all four possible combinations.

To screen for inserts on the third chromosome, the following scheme was developed. Twenty virgin females (Birm-2; ry^{506}) were mated with 10 males (iso-2; ry^{506}*Sb P*[ry^+ 2-3](99B)/TM6B, *Hu Dr*). These crosses were maintained at 25°C and transferred every 2 to 3 days, and the bottles containing larvae were transferred to 17°C to minimize subsequent sterility of the dysgenic males (4). Groups of one to three dysgenic male progeny of these crosses (Birm-2/iso-2; *Sb* ry^{506} *P*[ry^+ 2-3](99B)/ry^{506}) were each mated with 20 virgin females (TM3, *Sb Ser ry e*/kar^2 Df(3R)*ry*). Groups of 10–12 male progeny, the mutagenized males (Birm-2 or iso-2; TM3, *Sb Ser ry e*/ry^{506}), were each mated with 20 virgin females (TM3, *Sb Ser ry e*/kar^2 Df(3R)*ry*). These crosses were transferred after 3, 6, and 9 days and were used for screening as described below. W. Engels supplied the stocks Birm-2; ry^{506} and CyO/Sp; ry^{506}*Sb P*[ry^+ 2-3](99B)/TM6, *Ubx* from which the isogenic strains used in the mutagenesis were derived. W. Leiserson supplied the iso-2; *Sb* ry^{506} *P*[ry^+ 2-3](99B)/TM6B, *Hu Dr* isogenic lines, and L. Craymer supplied the TM6B chromosome (6).

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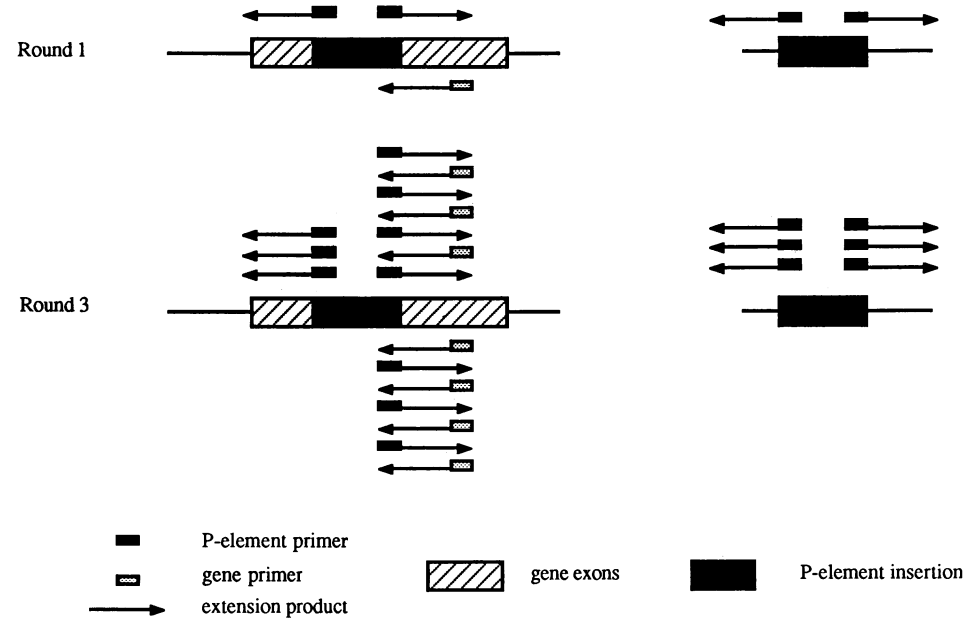

FIG. 1. Use of PCR to detect insertion of a *P*-element transposon into a specific gene. Two segments of genomic DNA are shown. In one case (*Left*), a *P*-element transposon is shown inserted near a gene of interest; in the other (*Right*), it is at a random unlinked site. Two oligonucleotide primers are shown, one containing a sequence from the gene to be targeted (▨), and the other containing the terminal sequence of the *P* element (■). (*Upper*) DNA extension products after one cycle of replication. (*Lower*) DNA extension products after three cycles. Exponential amplification occurs only when the newly synthesized strand initiated by each of the primers incorporates sequences complementary to the other—in this example, only when a *P* element has inserted near the gene oligonucleotide. Other DNA extension products increase only linearly and, after multiple rounds of amplification, represent only a small proportion of the total DNA synthesized (2).

Screening. At 6 days, parental males were removed from a set of five bottles and pooled (about 50 males total). Their DNA was isolated and screened for the presence of inserts as described in the text below. For the initial PCR screen to detect for the presence of inserts, two such DNA preparations were combined, representing a total of about 100 mutagenized males. When the presence of an insert was detected, each of the two DNA preparations was then tested separately. This procedure helped to eliminate false positives due to the occasional spurious occurrence of multiple amplification products when sets of oligonucleotides were used (see text for details).

Once the presence of an insert in the tested population was confirmed, the problem became to pinpoint an individual fly carrying it. From the five initially pooled bottles, ≈100 male and female progeny [TM3, *Sb Ser ry e/ry*^{506} or *kar*^{2} Df(3R)*ry*] were collected from each. While the males were maintained at 17°C, DNA was isolated from the females and checked for the presence of the insert. When the insert was found in the DNA of the females in a particular bottle, groups of 5 males held from that bottle were each mated with 20 virgin females [TM6B, *h D*^{3} *e*/In(3R) *Mo Sb sr*]. [Note: This cross is optional. It was included in these studies to provide an additional opportunity to confirm the presence of an insert. However, individual males could be mated at this point in the scheme.] From each of these crosses, ≈40 female and male progeny [TM6B, *h D*^{3} *e/ry*^{506} or *kar*^{2} DF(3R)*ry*] were collected. DNA was again isolated from the females and screened for the presence of the insert, while the males were held at 17°C. When the insert was found among the female progeny of a given cross, then individual males from that cross were mated, each with 15 virgin females [TM6B, *h D*^{3} *e*/In(3R) *Mo Sb sr*]. To obtain results rapidly, without waiting for the progeny from these crosses to mature, DNA was isolated from young larvae as described below and checked for the presence of the insert. Males and virgin females (TM6B, *h D*^{3} *e/ry*^{506}) from crosses that showed an insert were then mated to establish insert-containing lines.

DNA Extraction. Flies were collected in screw-top microtubes (Sarstedt) and stored at −70°C. With the plunger from a 1-ml syringe, they were mashed in 500 μl of buffer A (100 mM Tris chloride, pH 7.6/100 mM EDTA/100 mM NaCl/0.5% $NaDodSO_4$), and the "mashate" was incubated at 65°C for 30 min. It was made 1.2 M in KOAc by addition of 75 μl of 8 M KOAc, thoroughly mixed, chilled on ice for 20 min, and then centrifuged at 12,000 × *g* for 5 min at room temperature. The supernatant was removed and centrifuged at 12,000 × *g* for 5 min. That supernatant was carefully removed, mixed with an equal volume of 100% ethanol at room temperature, incubated for 5 min at room temperature, and centrifuged at 12,000 × *g* for 5 min. The DNA pellet was washed with 70% ethanol, dried, and resuspended overnight at 4°C in 325 μl of TE buffer (15 mM Tris chloride, pH 7.6/1 mM EDTA). After resuspension, the sample was centrifuged for 1 min at 12,000 × *g* to remove insoluble material. The supernatant was made 0.2 M in NaOAc, mixed with two volumes of 100% ethanol, incubated for 5 min at room temperature, and centrifuged at 12,000 × *g* for 5 min. The pellet was washed with 70% ethanol, dried, and resuspended in 100 μl of TE buffer. Larval DNA was made from embryos collected overnight and then aged for 24 hr at 25°C. The larvae were collected in about 50 μl of distilled water and frozen on dry ice. They were processed as described above except that they were lysed in 200 μl of buffer A, all other additions being proportional to this reduced volume, and the second ethanol precipitation was omitted.

PCR Reactions. The reaction volume of 20 μl included 0.5 units of *Taq* polymerase (Perkin–Elmer/Cetus), 0.5 μg of the DNA preparation, 200 μM each NTP (dATP, dCTP, dGTP, and dTTP), and 0.4 μM each oligonucleotide in a buffer containing 50 mM KCl, 10 mM Tris chloride (pH 8.3), 1.5 mM $MgCl_2$, 0.1% gelatin. The reaction mixtures were overlaid with mineral oil (Sigma), incubated for 5 min at 94°C, taken through 40 cycles of 1 min at 94°C/1.5 min at 55°C/3 min (plus an increase of 5 sec each cycle) at 72°C. After these cycles, the samples were incubated for an additional 7 min at 72°C. All

incubations used a Perkin–Elmer/Cetus thermal cycler. After cycling, 5 μl of 5X DNA sample buffer (1X = 0.25% bromophenol blue/0.25% xylene cyanole/15% Ficoll), was added to the sample, which was thoroughly mixed and then centrifuged at 12,000 × *g* for 10 sec; 20 μl of the sample was removed and analyzed on a 2% agarose gel. After electrophoresis at 100 V for about 4 hr, the gels were stained with ethidium bromide and photographed. For hybridization tests, the gels were transferred by standard procedures (7) to Hybond nylon filters (Amersham). After UV crosslinking, the filters were prehybridized for 10 min or more at 65°C in a hybridization buffer (8) containing 0.5 M sodium phosphate (pH 7.2), 1 mM EDTA, and 8% (wt/vol) $NaDodSO_4$. They were then hybridized for about 17 hr at 65°C with the cloned cDNA, labeled by primer extension of random hexanucleotides (9) in the presence of [^{32}P]dCTP (specific activity ≈ 0.5–3 × 10^8 cpm/μg), at 10^6 cpm/ml. The filters were washed three times for 20 min each at 65°C in 0.2× SSPE/0.1% $NaDodSO_4$ (1× SSPE = 0.15 M NaCl/10 mM NaH_2PO_4/1 mM EDTA, pH 7.4) and exposed to Kodak XAR-5 film for about 12 hr at −70°C with Cronex Lightning Plus intensifying screens.

RESULTS

Sensitivity of the Method. To test the feasibility of this technique to detect targeted insertions into the *Drosophila* genome, tests were first done with a mutation known to have been induced by the insertion of a defective *P* element into the vermilion gene (10). The nucleotide sequences of that gene (L.L. Searles, personal communication) and the terminal repeats of the *P* element (11) were used to design appropriate oligonucleotide primers. One fly containing the insertion was mixed with 100 wild-type flies lacking it. From each of 10 such groups of 101 flies, DNA was isolated and subjected to 40 cycles of PCR in the presence of the primers. In all 10 preparations, the predicted 180-nucleotide amplification product associated with the insert was seen; it was not seen in control DNA isolated from wild-type flies. The amplified product was abundant and easily visible on ethidium bromide-stained agarose gels. The reaction product, transferred to nylon membranes, gave a positive result when probed with a labeled vermilion gene clone (data not shown). This model system demonstrated the ability to detect reproducibly an insertion in 1 fly of 100.

One potential pitfall of PCR techniques is that spurious amplification products may arise when using multiple oligonucleotide primers. The sources of these spurious products are not known; some might arise, stochastically, by "self-priming" from sequences within the genome that are partially homologous to the primers. These background products are reduced but not eliminated by high-temperature incubation during hybridization and primer extension (2). Fig. 2 *Left* shows an example of 10 duplicate PCR reactions of normal DNA with a gene primer (a partial sequence of the 4A11 cDNA; see below) and the *P*-element primer (lanes 1–10). Spurious amplification products were prominent in 1 of 10 cases (lane 1). Similarly, of the 63 PCR reactions involved in the primary screen for insertions described below, about 10% gave significant levels of such products with this oligonucleotide mixture. Each set of oligonucleotides gives a characteristic set of such spurious products, aiding in their recognition. In addition, to eliminate false positives due to such spurious products, we combined two DNA preparations for each PCR reaction of the primary screen (see *Materials and Methods*). When amplification products were detected in a particular reaction, the two DNA samples were then tested separately. Failure to observe the same products indicated that they had been spurious. When one of the preparations confirmed the previous result, it was taken as evidence of a possible insert. In addition, the primary screen included a positive control reaction for the quality of each DNA preparation, using two primers that yield a 134-nucleotide amplification product (Fig. 2 *Left*, lane 11). Of the 126 DNA samples involved in the primary mutagenic screen, this product was observed in all except 2 cases.

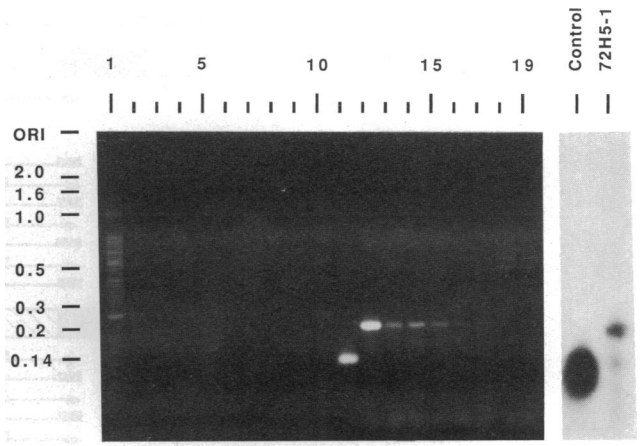

FIG. 2. Detection by PCR of an insertion into the 72H5 gene. (*Left*) Products of PCR reactions were separated on 2% agarose gels and stained with ethidium bromide. The size scale in kb is at the left. Lanes 1–10 show products of 10 individual control reactions with DNA from the unmutagenized parental strain using a 4A11 gene primer (5′-GAACCACTCGGGGATCGGAGGCCA-3′) and the *P*-element primer (5′-CGACGGGACCACCTTATGTTATTTCATCATG-3′). Variable amounts of spurious amplification products occurred; these were particularly prominent in lane 1 (see text). Lanes 11 and 12 show products of DNA from mutagenized flies homozygous for the 72H5-1 insertion. Lane 11 shows products of the positive control reaction using two primers from the 72H5 cDNA (5′-GGCGGTGGAGCAACTGGAGTGACTGC-3′ and 5′-CAAGTAGTTAGTCCTGCGGCAGTGGC-3′); these primers gave an amplification product of 134 nucleotides and were used to assess the quality of each DNA preparation of the primary screen. Lane 12 shows the product produced by using a pair of 72H5 gene primers (5′-GCAGTCACTCCAGTTGCTCCACCGCC-3′ and 5′-CAGTTGCACCGCCTGTCGAAGTTAC-3′) along with the *P*-element primer. The product indicates the existence of a *P*-element insertion about 200 nucleotides from one of the gene primers (two gene primers were used to increase the chance of detecting an insertion). Lanes 13–19 show the effect of progressive dilution with parental DNA of the DNA sample used in lane 12; the same primers were used. The dilutions were 1:10, 1:50, 1:100, 1:200, 1:400, 1:800, and 1:1600. (*Right*) PCR products from similar reactions to those shown at the left (lanes 11 and 12) were transferred to nylon and probed with the 72H5 cDNA clone. The control lane is a positive control as in lane 11. The 72H5-1 lane shows homology of the new PCR product with the cDNA; the relative weakness of this signal could be due to a less-than-complete extent of overlap with the cDNA—e.g., if the insert were in an intron.

Detection of Insertions. The procedure was then used to detect possible insertions into or near two genes of unknown function represented by cDNA clones. The first clone, 72H5, was isolated from a head cDNA expression library (12) by using a photoreceptor-specific monoclonal antibody as a probe (unpublished data). The second, 4A11, was isolated from a head cDNA library by a differential screening technique (3). The 72H5 cDNA recognizes a 3-kb transcript, and 4A11 recognizes a 1.5-kb transcript. Both poly(A)$^+$ transcripts are expressed in the adult compound eye; they show no detectable expression in "eyes absent" mutant flies, which lack the eyes. Gene primers for each cDNA were oligonucleotides 23 to 28 nucleotides long and with about 50% G+C content. The *P*-element primer was an oligonucleotide containing the entire 31 nucleotides of the inverted repeat of the transposon (11).

Flies containing random *P*-element insertions were produced by the dysgenic method of Robertson *et al.* (4), which

involves the introduction of very high levels of transposase activity for a single generation into a strain of flies carrying multiple defective *P* elements. Robertson *et al.* (4) observed an average of two *P*-element insertions per X chromosome per generation. The rate of two insertions per major chromosome arm would correspond to ≈10 new insertions per mutagenized genome. Since the *Drosophila* genome contains about 1.6×10^8 nucleotides, the average probability of insertion into a 2000-nucleotide segment would be on the order of 10^{-4} per fly, although *P*-element insertion sites are known to be nonrandom (13).

The primary screen for inserts involved testing DNA isolated from pools of about 100 mutagenized males by using various combinations of oligonucleotide primers. Primers for four different genes were used in the initial screen; of these, two yielded amplification products (see below). For any pool of flies for which the DNA gave an amplification product, the progeny were mated with flies carrying multiple third chromosomal inversions, their DNA was then isolated, and the PCR reactions were repeated. Lines of flies were progressively subdivided in this manner until a line of flies arising from an individual male was established.

Among 6316 mutagenized males tested, amplification products with gene primers from the 72H5 gene and the 4A11 gene were detected. In each case, amplification of DNA isolated from flies carrying these insertions (72H5-1 and 4A11-1) produced products that could be seen by ethidium bromide staining, as well as by transfer to nitrocellulose and probing with the labeled cDNA clone. In Fig. 2 *Left*, lane 12 shows the results for 72H5-1, as compared with the 134-nucleotide product of the control (lane 11). A single product of about 200 nucleotides indicated an insertion into the 72H5 gene. This figure also shows a reconstruction in which DNA from flies homozygous for insert 72H5-1 was progressively diluted with wild-type DNA (lanes 13–19). This and other reconstruction experiments showed that an amplification product may be routinely detected on ethidium bromide-stained agarose gels at a DNA dilution of 1 in 400. These data suggest that the primary screen, testing 100 mutagenized flies to detect a heterozygous insertion in any one of them, is well within the sensitivity of the technique.

To provide additional evidence that the 72H5 amplification product was indeed derived from the 72H5 gene, the 200-nucleotide product was run on a gel, transferred, and hybridized with labeled 72H5 cDNA, from which the sequence had been taken for use as the gene primer (Fig. 2 *Right*).

Results for the 4A11-1 amplification product are shown in Fig. 3. Two products of ≈620 and ≈500 nucleotides were seen. Each of the two products hybridized to the 4A11 cDNA clone (Fig. 3 *Right*). Nevertheless (see below), the origin of these products is not clear.

Confirmation of Insertions into the Genome at the Target Site. The presence of *P*-element inserts into or near the 72H5 gene (Fig. 4) was confirmed by *in situ* hybridization to the larval salivary gland chromosomes of the insertion strain by using labeled *P*-element DNA as probe. In addition, the 72H5-1 amplification product was gel isolated, labeled with biotinylated dUTP, and hybridized *in situ* to larval salivary gland chromosomes. The amplification product also hybridized to the same location as the cDNA (data not shown), confirming its origin at the 72H5 gene. When both labeled *P*-element DNA and labeled 72H5 cDNA were hybridized in the same reaction to chromosomes from the 72H5-1 insertion strain, only one hybridization band was observed at the site (data not shown).

In contrast, the 4A11-1 amplification product apparently did not arise from the 4A11 gene. There was no site of *P*-element hybridization in the insertion strain at the location of the 4A11 gene, chromosomal band 72E. Also, the 4A11-1 amplification product did not hybridize at this location. What

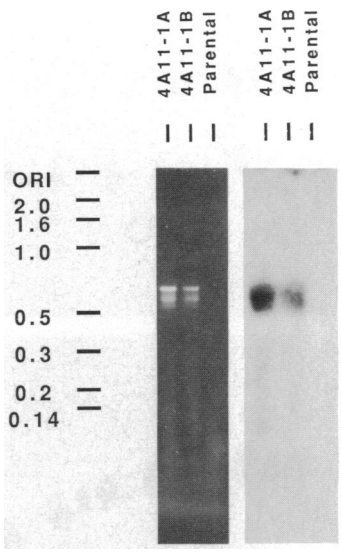

FIG. 3. Detection by PCR of amplification products with the 4A11 gene primer. (*Left*) Products of PCR reactions using the 4A11 gene primer plus the *P*-element primer (Fig. 2 legend) run on 2% agarose gels and stained with ethidium bromide. The size scale in kb is at the left. The DNA preparations used in the PCR reaction were two strains (A and B) homozygous for the insertion and the parental strain. Two prominent amplification bands were produced (see text). While derived from different bottles of flies in the initial mutagenic screen, 4A11-1A and 4A11-1B are likely to represent sibling progeny from the same premeiotic *P*-element insertion. (*Right*) The PCR products of a gel similar to *Left* were transferred to nylon and probed with the labeled 4A11 cDNA clone. Strong hybridization was observed with the amplification products of the two insertion strains but not with the reaction run with parental DNA.

gave rise to the 4A11-1 amplification product is not clear. It could be due to a *P*-element insertion near some other gene that contains sequences homologous to the 4A11 cDNA. In any case, this observation underscores the need to confirm the identity of PCR products by mapping them back to the targeted chromosomal site.

Mutant flies initially detected by the PCR technique are heterozygous for *P*-element insertions. Depending on the site of an insertion, it may fail to disrupt gene function sufficiently to cause a change in phenotype or it may partially or completely abolish gene activity, revealing aspects of the gene's function. To test for possible mutant phenotypes, the 72H5-1 insert was made homozygous. Homozygotes were recognized by their rosy colored eyes, due to the ry^{506} mutation present on the mutagenized chromosome, as well as by the lack of the dominant phenotype associated with the TM6B, *h* D^3 *e* balancer chromosome (see *Materials and Methods*). Preliminary analysis of these flies did not reveal any obvious phenotypes. Eye morphology and white-light countercurrent phototaxis (14) of flies homozygous for the 72H5-1 insertion were normal. In addition, the 72H5 monoclonal antibody (used to isolate the 72H5 cDNA) stained adult flies homozygous for the 72H5-1 insertion normally. This particular insertion might have relatively subtle phenotypes that may be revealed by closer analysis, or it may have occurred at a location having little effect on gene function. While the primers that detected the insertion were within or very close to protein coding sequences of the 72H5 cDNA, their relationships to possible intron/exon junctions are not yet known.

DISCUSSION

Given that the technique works, it will now be possible to isolate a larger set of insertions that may include null or strongly hypomorphic alleles that lack RNA or protein

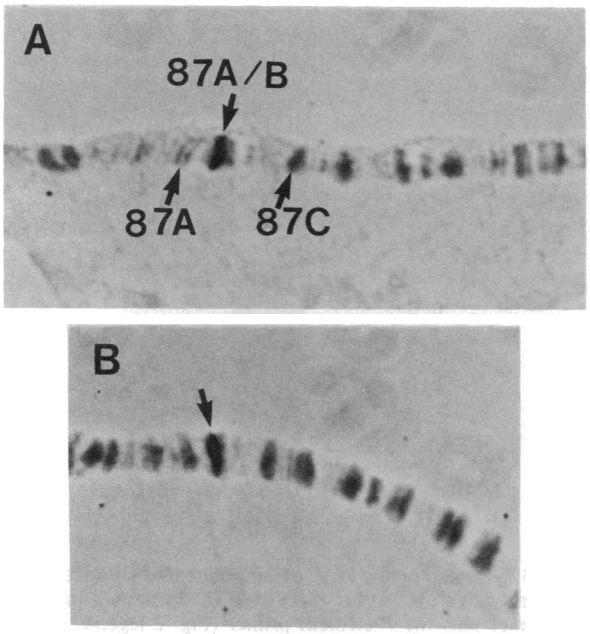

FIG. 4. The 72H5-1 insert strain has a *P* element at the location of the 72H5 gene. (*A*) Larval salivary gland chromosomes from Canton-Special normal flies hybridized with biotinylated 72H5 cDNA. The 72H5 gene was located in the region of three dark bands at 87A9; 87B1,2, and 87B4,5. These bands are generally poorly resolved during preparation of larval salivary gland chromosomes. (*B*) Hybridization of biotinylated *P*-element DNA to larval salivary gland chromosomes from a stock homozygous for the 72H5-1 insert. This stock had four sites of *P*-element hybridization (63 C/D; 86 B; 87 A/B; and 99 A/B), including the one shown at the site of the 72H5 gene. The PCR amplification product (not shown) also hybridized to the 72H5 gene site.

expression. In principle, insertions can be targeted, through the choice of appropriate primers, to sites that are most likely to disrupt function, such as 5′ regulatory regions (13). Alternatively, insertion strains obtained by this method can be used to isolate sets of deletions in the region of the gene. When taken through an additional round of hybrid dysgenesis, *P*-element insertions tend to undergo excision, sometimes deleting sizable segments of DNA flanking the insertion site (13). Such studies are currently in progress.

The mutagenesis scheme can be modified to use a *P*-element transposon carrying a dominant marker, such as *P* (w^+). This will make it easier to recognize when an insertion is present, even in the heterozygous form. Excision of the *P* element could be signaled by the loss of the dominant marker. In addition, a *P* element could be modified to be more mutagenic when inserted within a transcribed region. For instance, sequences causing polyadenylylation and termination of transcription could be introduced into a *P* element so that transcription would not proceed through it.

To eliminate the type of false positive represented by the 4A11-1 amplification product, an additional step should be added—namely, to gel-isolate the amplification product, label it with biotinylated dUTP, and hybridize it *in situ* to larval salivary gland polytene chromosomes to determine its location of origin. Any product not at the same location as the target gene would not be further pursued.

This paper describes a method of using PCR to detect *P*-element transposon insertions into or near a cloned gene, illustrated by one example. Several other methods have been used in *Drosophila* to isolate mutations in cloned genes. One, the mutational saturation of the region of a deficiency encompassing the gene (15), can be quite time-consuming and must be done separately for each gene. Another, transformation rescue of an existing mutation (16), depends on the fortuitous existence of a mutation in the region of the clone, with a reasonable guess at a possible mutant phenotype. A third, screening mutagenized flies directly for those that lack expression of a particular antigen (17), relies on the antigen having a favorable expression pattern and is practical only for nonessential genes. The method described here does not rely on prior knowledge of the function of the gene or the ability to interpret mutant phenotype. Primers from several different genes can be used to screen the same mutagenized population for insertions into any of the genes. In addition, such insertions can be detected in the heterozygous state, allowing for the isolation of recessive lethal mutations.

The experiments described here were performed with *Drosophila*. It is manifest that the same principle could be applied to a wide range of organisms, as well as to cells in culture by using appropriate vectors that become integrated into the genome.

Note Added in Proof. K. Kaiser and S. F. Goodwin, Glasgow University, (personal communication) have similarly used PCR to detect insertions into the singed gene of *Drosophila*.

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